Two new species of ergasilid copepods parasitic on fishes cultured in brackish water in Taiwan

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Abstract.—Ergasilus lobus and Diergasilus kasaharai are described based on the specimens obtained from the gill filaments of the moribund fishes cultured in brackish water in southern part of Taiwan. The former species was obtained from Malabar reef-cod (Epinephelus malabaricus) and the latter, from milkfish (Chanos chanos) and Borneo mullet (Liza macrolepis). A key to the 22 species of Ergasilus occurring on the coastal, brackish water fishes of the world is provided.

Copepods of the family Ergasilidae are generally known as parasites of freshwater fishes throughout the world. Nevertheless, some of them are found on estuarine and/ or coastal fishes. According to Ho (1991), in the course of copepod evolution, poecilostome copepods represented by the Ergasilidae succeeded only once in invading freshwaters, and those ergasilids occurring on coastal, brackish water fishes are a group of poecilostomes secondarily adapted for marine existence.

Several species of ergasilids are known to cause disease in finfish cultured in brackish waters (Nigrelli 1950, Nakajima & Egusa 1973, Paperna 1975, Wijeyaratne & Gunawardene 1988, Leong & Wong 1988, Hogans 1989). In this paper, two species of ergasilids are reported from the fishes cultured in brackish water in the southern part of Taiwan, a species of *Ergasilus* and a species of *Diergasilus*.

On 2 October 1992, a moribund Malabar reef-cod (*Epinephelus malabaricus*) was brought to the Laboratory of Fish Disease at the Chiayi Institute of Technology for examination. The fish came from a culture pond located in Chi-ku Village of Tainan County. A close examination showed that its gill filaments were infected with Ergasilus sp. (Fig. 1A) or contained many "vacuoles" (Fig. 1B). Since no other abnormalities were detected, the death of the fish was suspected to be caused by the infection of the ergasilid copepod. Later, on 23 June 1993, a mass mortality occurred in another culture pond in the same village where about 9000 juvenile Malabar reef-cods (imported from Thailand) were cultured. For 15 days, about three to four hundred dead fish were removed daily from the pond. Examination of the dead fish revealed the same condition, carrying Ergasilus sp. and with many "vacuoles" in the gill filaments. as observed in October 1992 from another pond. A subsequent histological examination of the "vacuoles" in the gill filaments showed no trace of microbes or protozoan parasites; "vacuoles" may have formed by the host's reaction to the hooking and/or penetration of the ergasilid's antenna.

On 23 December 1993, a mass mortality occurred in a culture pond in Chi-ku Village where about 20,000 milkfish (*Chanos chanos*) were cultured. In the beginning, about 10 fish died daily, but after a week more than 100 fish died in a day. Examination of the moribund fishes revealed that



Fig. 1. Gill filaments of diseased fishes. A. from *Epinephelus malabaricus* showing attachment of *Ergasilus lobus*; B. from *E. malabaricus* showing the "vacuoles"; C. from *Liza macrolepis* showing a heavy infestation with *Diergasilus kasaharai*; D. from *L. macrolepis* showing attachment of *D. kasaharai*.

death was caused by the parasitism of a species of ergasilid copepod belonging to Diergasilus. More than 130 copepods could be removed from the gill filaments of an infected fish. In October 1994, the same parasite was found on Borneo mullet (Liza macrolepis) cultured together with tilapia (Oreochromis sp.) in a pond in Hu-Nei Village in Kaoshiung County. Two to three hundred mullets per day died for about a week. The moribund mullets, swimming at the edge of the pond, were found to carry between 500 to 1000 copepods on their gill filaments (see Fig. 1C); these filiments showed inflamation, necrosis and were coated with excessive amount of mucus. Undoubtedly, the death of the mullets were due to the heavy parasitism of Diergasilus sp. (Fig. 1D).

About 160 species of poecilostome copepods are currently classified in 26 genera of the family Ergasilidae (Malta 1993, 1994; Amado et al. 1995). Of these 26 ergasilid genera, *Ergasilus* is the largest with about three-quarters of the known ergasilid species. Identification of the species of *Ergasilus* has been a problem for many biologists who are not familiar with this group of parasitic copepods.

In as much as the culture of marine finfish in the coastal area in brackish water is becoming more and more popular in many parts of the world, identification of those potential fish disease causing ergasilids is becoming more and more indispensable. In addition to describing the above-mentioned species of *Ergasilus* and *Diergasilus* a key to the species of *Ergasilus* which may occur on coastal, brackish water fishes is also provided as a quick identification method for these pathogens.

Materials and Methods

Moribund fishes obtained from fishermen were examined under the dissection microscope for abnormalities and the presence of parasites. Upon the discovery of parasitic copepods, photographs were taken with the parasites in situ, after which the parasites were removed from the host and preserved in 70% ethyl alcohol. Microscopical examination of the ergasilid copepods was based on specimens fixed and preserved in ethyl alcohol and which were cleared in 85% lactic acid for a couple of hours before taking measurements and making dissections. All drawings were made with the aid of a camera lucida. All measurements were taken from the longest, widest and deepest parts of the body and are given in mm unless mentioned otherwise.

Descriptions

Ergasilus lobus, new species (Figs. 2-3)

Material examined.—10 ovigerous females recovered from gill filaments of a moribund Malabar reef-cod, *Epinephelus* malabaricus (Bloch et Schneider), cultured in Chi-ku Village in Tainan County in Taiwan on 23 Jun 1993. Holotype (USNM 278225) and 2 paratypes (USNM 278226) have been deposited in the Division of Crustacea, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Female.—Body 0.55 (0.53–0.57) long and 0.30 (0.27–0.32) wide, with greatly inflated cephalothorax (including first pediger) and relatively short and small metasome and urosome (Fig. 2A, B). Genital double somite distinctly wider than long (Fig. 2C) and armed on ventral surface with a row of fine spinules across mid-region and another row on posterior margin. Spinules on other parts of urosome as shown in Fig. 2D. Caudal ramus (Fig. 2C, D) slightly longer than wide, tipped with 1 long and 3 short setae. Egg sac (Fig. 2A) distinctly longer than body, about 0.64 in length and 0.16 in width.

Antennule (Fig. 3A) 6-segmented, armature of 3, 12, 4, 4, 2 and 7 elements. Antenna (Fig. 3B) strongly curved, without sensilla or seta; 1st segment wider than long, second segment slightly longer than

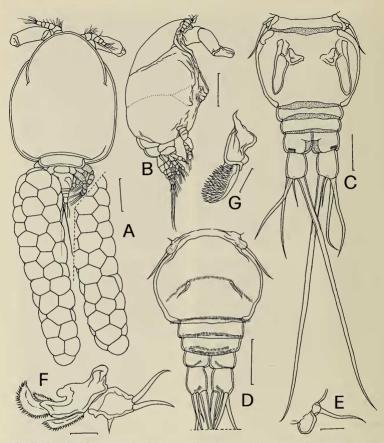


Fig. 2. Ergasilus lobus, new species, female: A. habitus, dorsal; B. habitus, lateral; C. urosome, ventral; E. leg 5; F. mandible and maxillule; G. maxilla. Scale bars: 0.1 mm in A, B; 0.03 mm in C, D; 0.01 mm in E, F, G.

third segment, and terminal claw distinctly shorter than third segment. Mandible (Fig. 2F) a serrated, falciform blade bearing a spinulose process on anterior margin and another uniserrated process on posterior margin. Maxillule (Fig. 2F) with 2 long and 1 short setae. Maxilla 2-segmented; proximal segment large and unarmed, distal segment (Fig. 2G) small and spinulose. Legs 1-4 (Fig. 3C-E) biramous, with formula of spines and setae as follows:

P1 Coxa 0-0 Basis 1-0 Exopod I-0; 0-1; II, 1, 4

Endopod 0-1; 0-1; II, 4

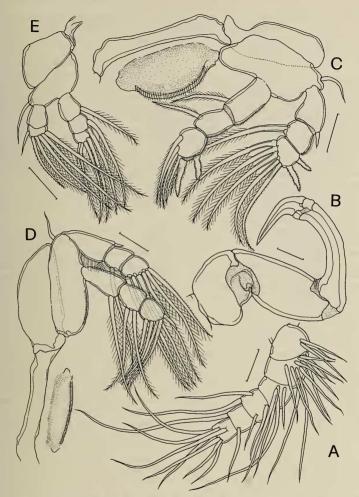


Fig. 3. *Ergasilus lobus*, new species, female: A. antennule; B. antenna; C. leg 1; D. legs 2 and 3; E. leg 4. Scale bars: 0.02 mm in A, C, D; 0.03 mm in B, E.

P2, P3 Coxa 0-0 Basis 1-0 Exopod 1-0; 0-1; 1, 5 Endopod 0-1; 0-2; 1, 4 P4 Coxa 0-0 Basis 1-0 Exopod 0-0; 1, 1, 4 Endopod 0-1; 0-2; 1, 3

Intercoxal bar with prominent posteroventral plate in leg 1 (Fig. 3C) less developed in legs 2 and 3 (Fig. 3D) and absent in leg 4. Leg 5 (Fig. 2C, D, E) much reduced, represented by a small knob tipped with a seta; a small protuberance located ventral to leg 5 (Figs. 2D, E).

Etymology.—The species name *lobus* in Latin means "a protuberance". It is here used as a noun in apposition and refers to the small rounded projection located ventral to the extremely reduced leg 5.

Remarks.-Currently, about 120 species of Ergasilus are known. However, only ten of them are characterized by a greatly inflated cephalothorax which is more than twice the length of the rest of the body. These ten species are: E. argulus Cressey, 1970: E. auritus Markewitsch 1940: E. centrachidarum Wright, 1882; E. luciopercum Henderson, 1926; E. manicatus Wilson, 1911: E. myctarothes Wilson, 1913: E. orientalis Yamaguti, 1939; E. parvitergum Ho, Jayarajan & Radhakrishnan, 1992; E. plecoglossi Yamaguti, 1939; and E. rotundicorpus Jones & Hine, 1983. The new species can be easily distinguished from these similar species, except for E. argulus, E. myctarothes, and E. parvitergum, by the absence of an inflated, cuticular, outer membrane between the first and second segments of the antenna.

According to Cressey & Collette's (1970) description, *E. argulus* differs from the new species in having two setules on the inner margin of the second segment of the antenna, a 2-segmented endopod on leg 1, and seven elements on the terminal segment of the exopod on legs 2 and 3. Based on Wilson's (1913) description, *E. myctarothes* is distinguishable from the new species by the fine structure of the antenna; its shaft bears a small, subterminal, inner protuberance and the claw is armed with two small teeth on inner margin near the center; besides, the armatures on legs 1 to 4 also show differences.

The new species is most similar to *E. parvitergum* known from India (Ho et al. 1992). The similarities are seen not only in the general appearance of the body, but also in the reduction of the tergum of the fourth pediger and leg 5. Nevertheless, *E. parvitergum* can be distinguished from the new species in lacking a lobe on leg 5 and having an outer spine on the second exopod segment of leg 1 and the first exopod segment of leg 4.

Diergasilus kasaharai Do, 1981 (Figs. 4-6)

Material Examined.—Numerous ovigerous females and young females recovered from gill filaments of milkfish (Chanos chanos) cultured in Chiku Village in Tainan County, Taiwan on 23 Dec 1993 and Borneo mullets (*Liza macrolepis*) cultured in Hu-Nei Village in Kaoshiung County, Taiwan in Oct 1994. Two lots of specimens (USNM 278227 and 278228) have been deposited in the Division of Crustacea, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Female.-Body 0.59 (0.51-0.68) long, 0.30 (0.23-0.44) wide, and 0.27 (0.16-0.33) thick, with greatly inflated cephalothorax (Fig. 4A, B). Cephalothorax (including first pediger) distinctly wider than long and truncated anteriorly. Metasomal somites abruptly narrowed from cephalothorax and decreasing in size posteriorly (Fig. 4A). Genital double somite wider than long, bearing a hvaline lateral spine in egg-sac attachment area (Fig. 4C). Spinulation on ventral surface of urosome as shown in Fig. 4C. Caudal ramus (Fig. 4C) about as wide as long and tipped with 1 long and 3 short setae. Egg sac (Fig. 4A, B) shorter than body, 0.51 (0.45-0.62) long and 0.11 (0.11-0.13) wide (based on 20 individuals). Antennule (Fig. 4D) 5-segmented, ar-

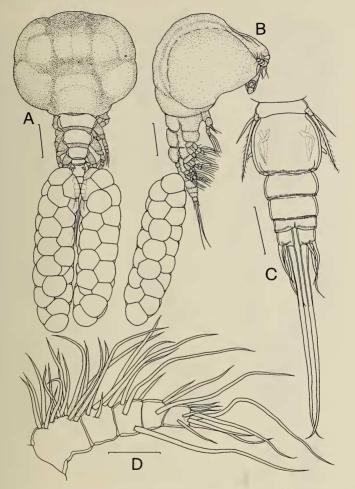


Fig. 4. Diergasilus kasaharai, female: A. habitus, dorsal; B. habitus, lateral; C. urosome, ventral; D. antennule. Scale bars: 0.1 mm in A, B; 0.04 mm in C; 0.03 mm in D.

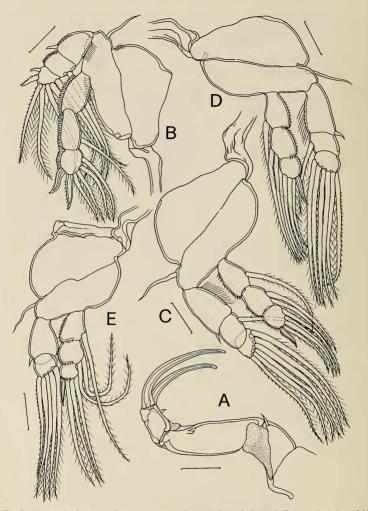


Fig. 5. *Diergasilus kasaharai*, female: A. antenna; B. leg 1; C. leg 2; D. leg 3; E. leg 4. Scale bars: 0.03 mm in A; 0.02 mm in B, C, D, E.

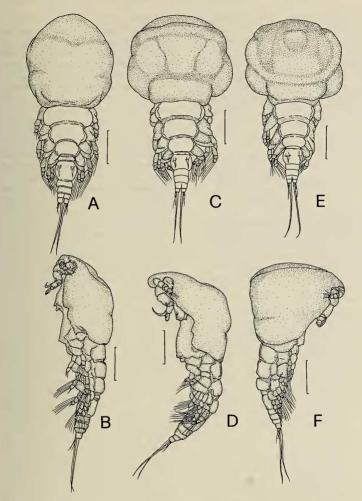


Fig. 6. *Diergasilus kasaharai*, habitus of females showing various state of inflation in cephalothorax: A. dorsal; B. same individual, lateral; C. dorsal; D. same individual, lateral; E. dorsal; F. same individual, lateral. Scale bars: 0.1 mm in all drawings.

mature of 15, 5, 4, 3 and 8 elements. Antenna (Fig. 5A) 3-segmented and tipped with 2 long, unequal claws; each segment bearing a small distal, inner seta; and second segment largest, longer than first and third segments combined. Mandible, maxillule, and maxilla as in the above species. Legs 1-4 (Fig. 5B-E) biramous, with formula of spines and setae as follows:

P1	Coxa 0-0	Basis 1-0	Exopod	I-0; 0–1; II, 1, 4
			Endopod	0-1; 0-1; II, 4
P2, P3	Coxa 0-0	Basis I-0	Exopod	I-0; 0-1; 6
			Endopod	0-1; 0-2; I, 3
P4	Coxa 0-0	Basis 1-0	Exopod	I-0; 1, 4
			Endopod	0-1; 0-2; I, 3

Lateral margins of all endopods with a row of spinules, except for leg 1 with a row of teeth. First two exopodal segments of leg 1 with row of teeth on lateral margin. Leg 5 (Fig. 4C) much reduced, represented by a basal seta and a small papilla tipped with a long pinnate seta.

Remarks.—This is the second report of Diergasilus kasaharai. The first report was made by Do (1981) from the striped mullet (Mugil cephalus) caught in Kojima Bay, Okayama, Japan. The specimens from Taiwan bear close resemblance with those found in Japan in the structure of all appendages and differs from it only in the shape of the cephalothorax. In those specimens from Taiwan, the inflated cephalothorax is truncated at the front end (Fig. 4A, B), but in those from Japan, it is bluntly pointed as in a typical ergasilid copepod (Do 1981).

After a close examination of more than 100 individuals, it became clear that the shape and size of the cephalothorax of the specimens from Taiwan change as the parasite approaches the ovigerous state. It swells into a globose, lobular structure with a truncated anterior surface (Fig. 6A-C). And, even in the least inflated individuals (Fig. 6A, B), the cephalothorax is still distinguishable from those reported from Japan; it is not as pointed and globular as illustrated by Do (1981). However, with the lack of information about the maturity related changes in the cephalothorax of the Japanese specimens, we shall refrain from considering the above-mentioned differences as a species distinction between the specimens from Japan and Taiwan.

Key to Ergasilus Found on Coastal Fishes

There are more than 120 nominal species of *Ergasilus*. Most of them were either not well described by their discoverer, or have not been seen again since the original report. Further, their type specimens are either inaccessible or no longer extant. Consequently, it is difficult, if not impossible, to construct a key for quick identification of the members of this important genus of parasites. Fortunately, only about one-fifth of the members of *Ergasilus* are parasitic on the coastal, brackish water fishes and they are relatively better known; thus, attempt to construct a key to the species of *Ergasilus* in coastal waters is feasible.

Based on the reports of Wilson (1913), Brian (1927), Markewitsch (1933, 1940), Bere (1936), Yamaguti (1939), Redkar et al. (1952), Cressev & Collette (1970), Roubal (1981), Byrnes (1986), Kabata (1986, 1988, 1992), Leong & Wong (1988), Wijeyaratne & Gunawardene (1988), Ho et al. (1992), and the present work, 25 species of Ergasilus are currently known to occur on fishes of coastal, brackish waters, However, Ergasilus ponticus Markewitsch 1940 and Ergasilus wilsoni Markewitsch 1933 are excluded from the following key due to the lack of complete species information. Both of them were poorly described originally and have not been adequately redescribed.

Ergasilus funduli Krøyer 1863 was once considered to be a junior synonym of *Ergasilus manicatus* Wilson 1911 (Roberts 1970, Margolis & Arthur 1979). However, according to Kabata's (1986) re-examination of the type specimens (deposited in the Zoological Museum, University of Copen-

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hagen), the structure of the antenna of *E. funduli* clearly indicated that it is a different species. Nevertheless, details of the leg armature of *E. funduli* are still unknown, and it is not included in the following key.

While many of the 22 species appearing in the following key are known from a single location, some of them are widely distributed, for instance, Ergasilus orientalis Yamaguti has been reported from Japan (Yamaguti 1939), Australia and Brazil (Cressev & Collette 1970) and Ergasilus lizae Krøver is know from the Gulf of Mexico (Bere 1936), Pacific coast of North America (Hanan 1976, Kabata 1988), Australia (Kabata 1992), and the Mediterranean Sea (Ben Hassine 1983, Ben Hassine & Raibaut 1981). When a key is available for general use, more of these coastal Ergasilus species will show a pattern of much wider distribution than it is known now.

Appendages of small, difficult to dissect species of *Ergasilus* reported in the early part of this century were not well described; these appendages are the key characteristics to the species identification. In some cases, like Fraser's (1920) description on *Ergasilus turgidus*, there are discrepancies on the armature of appendages between the text and the illustrations. Thus, in construction of the following key, the characteristics of less ambiguity were employed and, in the case of conflict between the text and the illustration, the feature appeared in the illustration was adopted.

An interesting feature about the Ergasilidae is that only the adult female is parasitic. As in a typical free-living copepod, all members of this family pass through their naupliar and copepodid stages in a free-living mode of life; after molting into the adult and mating, the male dies and only the female seeks fish host to enter into a parasitic mode of life. Thus, the following key is intended only for the adult female.

 1a. Cephalothorax greatly inflated, at least twice longer than remaining body length (metasome + urosome) 2

b.	Cephalothorax may or may not be in-
	flated, if inflated less than twice length
	of remaining body (metasome + uro- some) 8
2a.	Antenna with an inflated membrane
2a.	between first and second segment 3
b.	Antenna without such membrane 6
3a.	Antenna with a balloon-like cuticular
	inflation at base of third segment of
	antenna manicatus Wilson, 1911
b.	Antenna without such inflation 4
4a.	Claw and shaft (third segment) of an-
	tenna with protuberance on inner mar-
	gin auritus Markevich, 1940
b.	
£ .	such protuberance
5a.	Cephalothorax twice longer than wide; ventral surface of caudal ramus
	with two rows of minute spines
	orientalis Yamaguti, 1939
b.	Cephalothorax about 1.5 times longer
	than wide; ventral surface of caudal
	ramus without spinules
	rotundicorpus Jones & Hine, 1983
6a.	Second segment of leg 1 exopod and
	first segment of leg 4 exopod with out-
	er spine 7
b.	Same segment on same leg ramus
_	without outer spine lobus, new species
7a.	Terminal segment of endopod on legs
	2 and 3 with six setae
b.	myctarothes Wilson, 1913 Same segment on same leg rami with
υ.	one spine and four setae
	parvitergum Ho, Jayarajan, &
	Radhakrishnan 1992
8a.	Middle segment of leg 2 endopod with
	one inner seta
b.	
	two inner setae 12
9a.	Middle segment of leg 3 endopod with
	one inner seta intermedius Kabata, 1992
b.	Middle segment of leg 3 endopod with
	two inner setae 10
10a.	Middle segment of leg 1 exopod with-
	out inner seta monodi Brian, 1927
b.	Middle segment of leg 1 exopod with
11a.	one inner seta 11
1 I a.	Antenna with an inflated membrane between first and second segments, its
	claw and shaft (third segment) bearing
	protuberance on inner margin
	turgidus Fraser, 1920

25

b.	Antenna without such inflated mem-
	brane or protuberance
	polynemi Redkar, Rangnekar &
	Murti, 1951
12a.	Antenna with an inflated membrane
	between first and second segments, its
	claw bearing two protuberances on in-
	ner margin
b.	Antenna without such inflated mem-
	brane or protuberance 13
13a.	Armature on terminal segment of leg
1 <i>5a</i> .	1 endopod II, 4 14
b.	Armature on same segment of same
υ.	leg 1, 5mugilis Vogt, 1877
c.	Armature on same segment of same
	leg I, 4longipalpus Wilson, 1913
14a.	Armature on terminal segment of ex-
	opod on legs 2 and 3 I, 6 15
b.	Armature on same segment of same
	leg rami 6 17
15a.	Middle segment of leg 1 exopod with-
	out outer spine 16
b.	Same segment of same leg with outer
	spine
	ceylonensis Fernando & Hanek, 1973
16a.	Antennule 6-segmented; eggs in egg
	sac multiseriate ogawai Kabata, 1992
ь.	Antennule 5-segmented; eggs in egg
υ.	sac uniseriate
	Radhakrishnan, 1992
17.	
17a.	Terminal segment of endopod on legs
	2 and 3 armed with one short and four
	long setae
b.	Same segment of same leg rami with
	five long setae
	borneoensis Yamaguti, 1954
18a.	Antennule 5-segmented 19
b.	Antennule 6-segmented 20
19a.	Caudal ramus long, ratio of length to
	width greater than 2; leg 1 intercoxal
	plate heavily armed with coarse spi-
	nules spinilaminatus Kabata, 1992
h	Caudal ramus short, ratio of length to
0.	width less than 1.5; leg 1 intercoxal
	plate without coarse spinules
20.	Radhakrishnan, 1992
20a.	Intercoxal plate of leg 1 with coarse
	denticles on posterior margin; proto-
	pods of legs 1-4 bearing patches of

b. Intercoxal plate of leg 1 without den-

ticles; protopods of legs 1-4 without patches of spinules ... *lizae* Krøyer, 1863

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